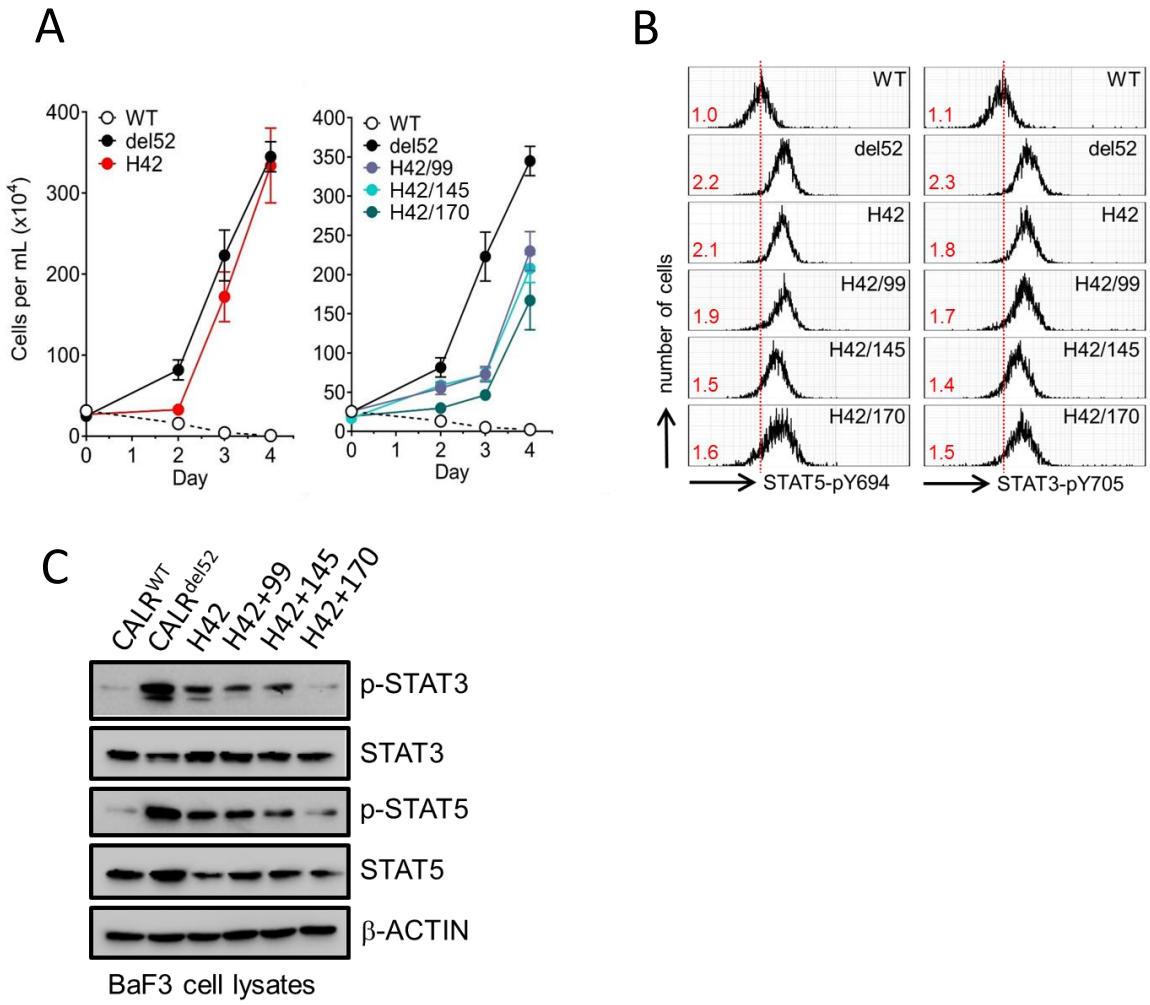
SUPPLEMENTAL INFORMATION

Zinc-dependent multimerization of mutant calreticulin is required for MPL binding and MPN pathogenesis

Jeanne F Rivera[1,2,11], April J Baral[1,11], Fatima Nadat[1], Grace Boyd[3], Hershna Patel[4], Rachael Smyth[1], Emma L Burman[1], Ghadah Alameer[1], Sally A Boxall[1], Brian R Jackson[1], E. Joanna Baxter[5], Peter Laslo[2], Anthony R Green[5,6,7], David G. Kent[3], Ann Mullally[8,9,10], Edwin Chen[1,4]

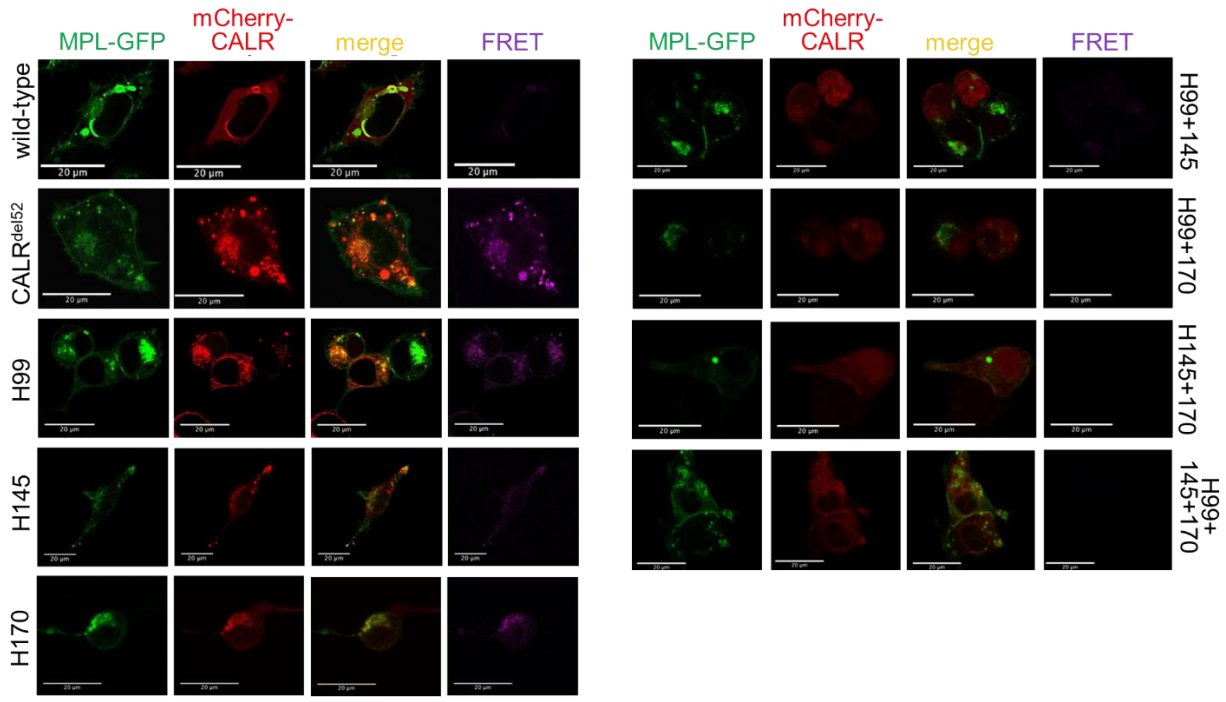
[1]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, United Kingdom.
[2]Division of Haematology and Immunology, Leeds Institute for Medical Research, St. James's University Hospital, University of Leeds, Leeds, LS9 7TF, United Kingdom.
[3]Department of Biology, University of York, York, YO10 5DD, United Kingdom.
[4]School of Life Sciences, University of Westminster, London, W1W 6UW, United Kingdom.
[5]Department of Hematology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge CB2 0QQ, United Kingdom.
[6]Wellcome MRC Cambridge Stem Cell Institute, University of Cambridge, Hills Road, Cambridge, CB2 0XY, United Kingdom.
[7]Department of Hematology, University of Cambridge, CB2 0XY, United Kingdom.
[8]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.
[9]Broad Institute, 415 Main Street, Cambridge, Massachusetts 02142, USA.
[10]Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, USA.
[11]These authors contributed equally to this work.

Figure S1



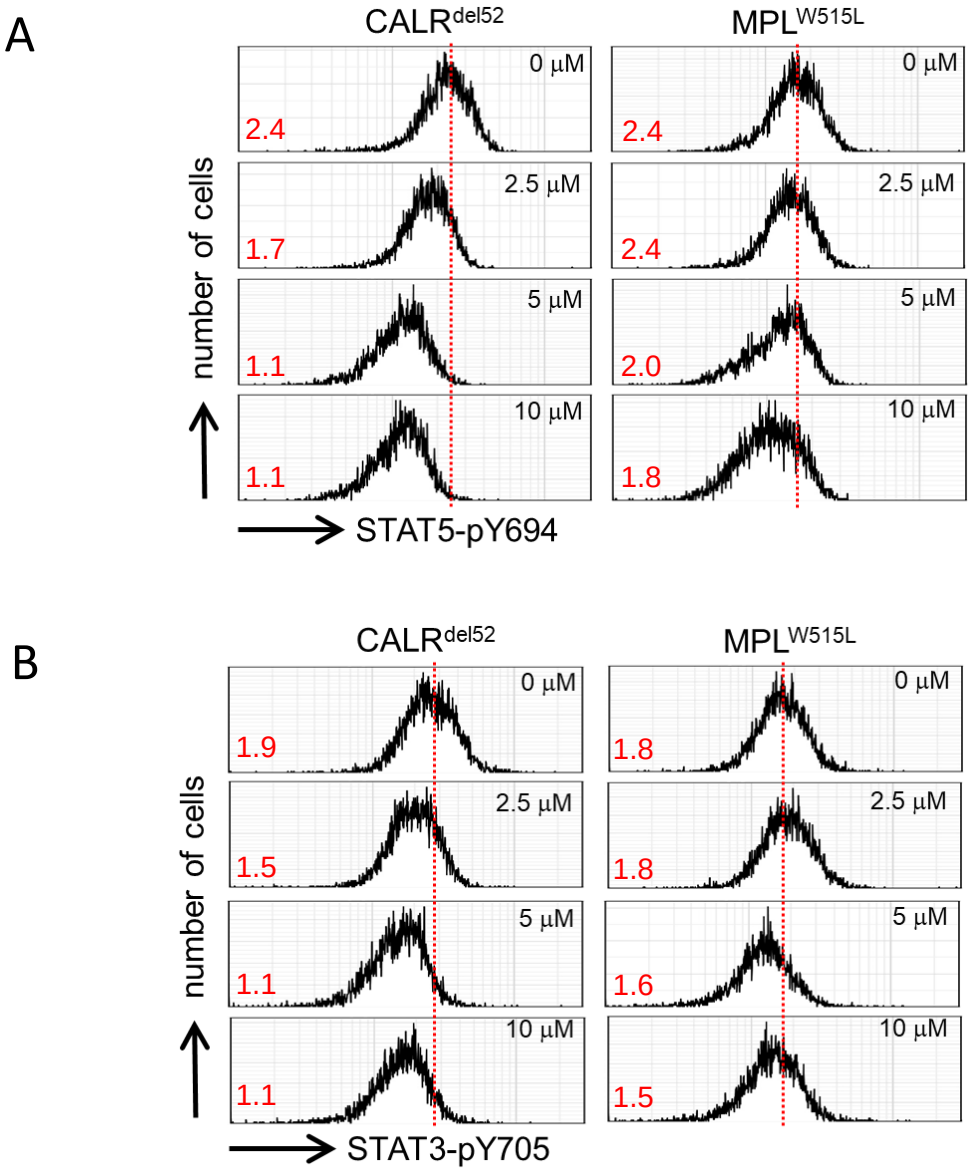
Supplemental Figure S1. Loss of H42 does not affect CALR^{del52} activity. (A) Growth curves in Ba/F3-MPL cells expressing CALR^{del52} variants harboring loss of histidine-42 (H42) alone or in combination with H99, H145 or H170. **(B-C)** Intracellular phosphorylation flow cytometry (Panel B) and immunoblotting (Panel C) demonstrate robust Stat3 and Stat5 phosphorylation in of Ba/F3-MPL cells expressing CALR^{del52} variants harboring loss of H42 alone or in combination with H99, H145 or H170. Numbers in red indicate ratio of mean fluorescence intensity for each sample relative to isotype control.

Figure S2



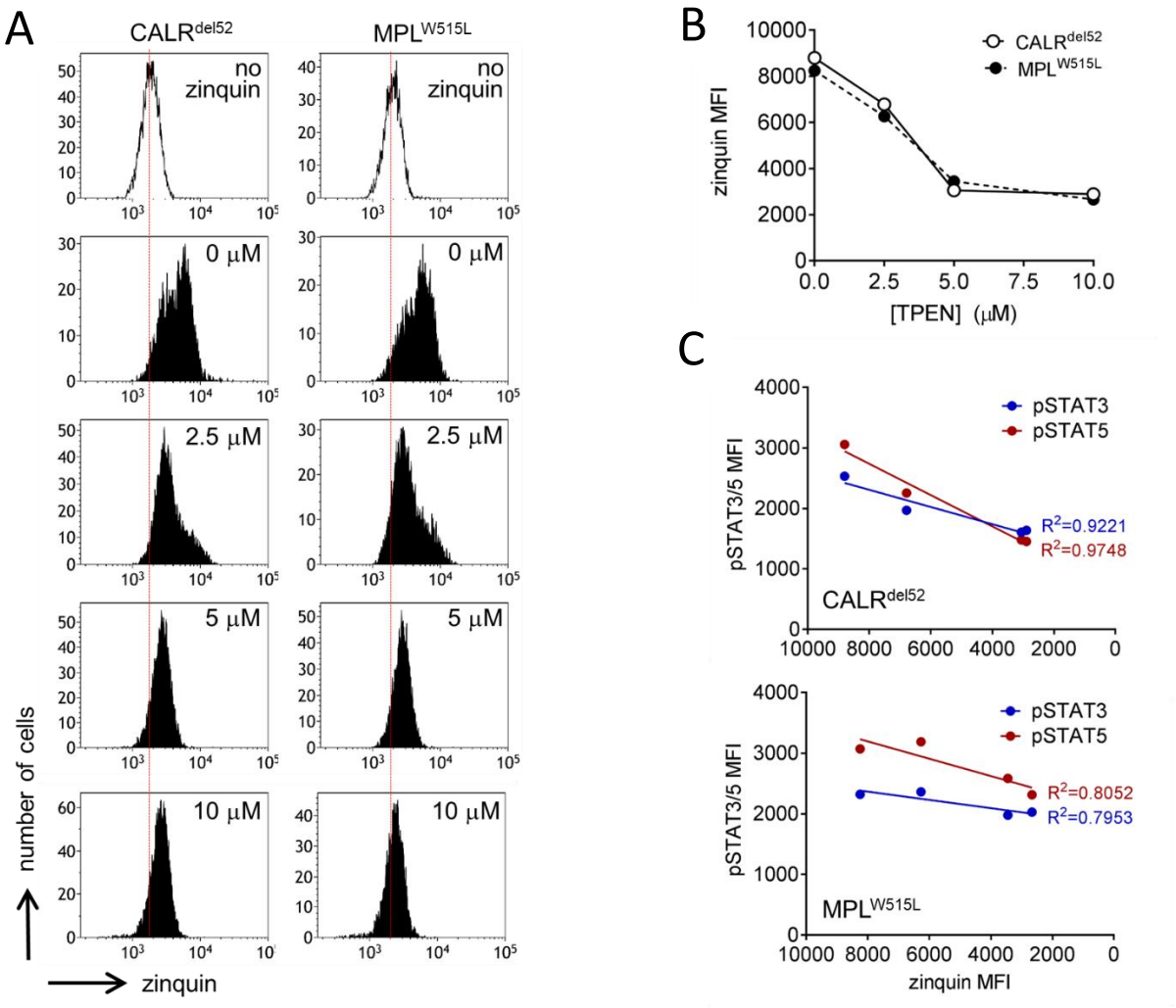
Supplemental Figure S2. Quantitation of CALR^{del52} and MPL co-localization by FRET. Representative confocal images of 293T cells expressing MPL-GFP fusion protein (green), mCherry-fused histidine-deficient CALR^{del52} variants fused to mCherry fluorophore (red) and areas of signal overlap (yellow). Quantitation of energy transfer by FRET are denoted in pseudocolour (magenta).

Figure S3



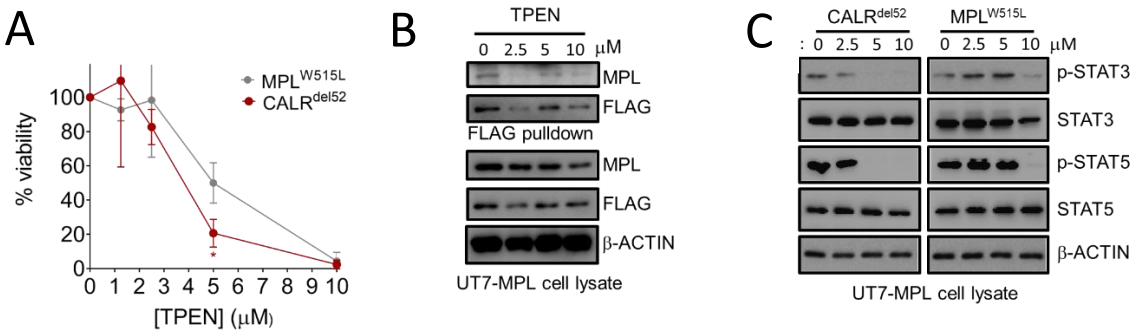
Supplemental Figure S3. TPEN abrogates CALR^{del52}-induced JAK-STAT signaling in Ba/F3-MPL cells. Intracellular phosphorylation flow cytometry demonstrate decreased Stat5 (Panel A) and Stat3 (Panel B) phosphorylation in Ba/F3-MPL cells in CALR^{del52} variants following treatment of TPEN for 4 hours. Numbers in red indicate ratio of mean fluorescence intensity for each sample relative to isotype control. The data are representative of 2 independent experiments.

Figure S4



Supplemental Figure S4. TPEN abrogation of JAK-STAT signaling correlates with decreased levels of intracellular zinc. (A) Zinquin staining quantitation of free intracellular zinc levels in TPEN-treated CALR^{del52} and MPL^{W515L} Ba/F3 cells by flow cytometry. **(B)** Correlation between zinquin mean fluorescence intensity (MFI) and TPEN dosage reveals similar extent of zinc chelation in both CALR^{del52} and MPL^{W515L} Ba/F3 cells. **(C)** Correlation between zinquin MFI and pSTAT3/5 MFI reveals strong correlation between zinc chelation and STAT3/5 signaling in CALR^{del52}-expressing Ba/F3-MPL cells and weaker correlation in Ba/F3-MPL^{W515L} Ba/F3 cells.

Figure S5



Supplemental Figure S5. TPEN abrogates CALR^{del52}-induced JAK-STAT signaling in UT7-MPL cells. (A) Viability of UT7-MPL cells expressing CALR^{del52} or MPL^{W515L} following treatment with TPEN for 48 hours. Cell viability was quantified by MTT assays. Each point represents the mean of three independent cultures. The data is representative of at least 2 independent experiments. Testing for statistical significance was performed using a student's t-test (*: $p<0.05$). **(B)** FLAG-pulldown assays demonstrating TPEN treatment disrupts CALR^{del52}-MPL binding in UT7-MPL cells. **(C)** Immunoblotting demonstrates decreased Stat3 and Stat5 phosphorylation status following TPEN treatment in CALR^{del52}-expressing UT7-MPL cells but not in UT7-MPL^{W515L} cells.